2.2. Experimental Test of Microbial Biocontrol of Cheatgrass

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2.2.1. Introduction

The establishment of alien annual grasses in disturbed Palouse and canyon grasslands presents a major challenge for parties seeking to restore native bunchgrass vegetation (Youtie 1997; Youtie et al. 1998, 1999; **Section 3, this volume**). One promising area of research involves manipulating the microbiota of the rhizosphere (the portion of the soil dominated by plant roots) to control weeds. (See Metting 1993 for review.)

Rhizobacteria (bacteria that actively colonize roots) with deleterious effects on weedy species have the potential to control weeds without the undesirable effects associated with the application of herbicides (Cherrington and Elliott 1987; Kremer 1987, Kremer et al. 1990; Kennedy et al. 1991; Kennedy and Kremer 1996). They are highly selective in their phytotoxic effects, and their use does not entail application of synthetic chemicals. Studies on agar, in growth chambers, and in the field have demonstrated that bacteria of the genus *Pseudomonas* have negative effects on the growth of cheatgrass (*Bromus tectorum*) (Cherrington and Elliott 1987; Kennedy et al. 1991).

Cheatgrass, a native of Eurasia, is ubiquitous in croplands and rangelands throughout the West (Mack 1981; Morrow and Stahlman 1984). Recruitment is usually concentrated in late summer and fall (Mack and Pyke 1983). Its ability to extract moisture from the upper layers of soil in winter and spring makes it an effective competitor for water (Hulbert 1955; Evans et al. 1970).

Methods of controlling cheatgrass are non-selective (Morrow and Stahlman 1984). Cheatgrass control is especially problematic in areas where maintenance or restoration of native grassland vegetation is the goal. There is no herbicide that is specific for cheatgrass, and burning can cause unacceptable mortality in native bunchgrasses and soil crust species (Youtie et al. 1999). In this study, we tested the effectiveness of *Pseudomonas fluorescens* as a biological agent to control cheatgrass on a formerly cultivated site where a program to restore native perennial grasses is in progress.

2.2.2. Methods

We tested the effects of *Pseudomonas* on germination and emergence of cheatgrass in a portion of a field in Pullman, Washington. The site was cultivated until 1995, when efforts to restore native grasses and forbs were initiated. The site was seeded with bluebunch wheatgrass (*Pseudoroegneria spicata* ssp. *spicata* [=*Agropyron spicatum*]) and Idaho fescue (*Festuca idahoensis*) in the fall of 1996 and again in the fall of 1997, but coverage of native perennial grasses remained low, in part because of a heavy infestation of cheatgrass.

After the onset of the fall rains in 1999, we inoculated the treatment areas with a culture of *Pseudomonas fluorescens* strain D7 at a concentration of 10^9 cells per plot. Because of the uniformly high density of cheatgrass on the study site prior to the start of the experiment, we assumed that the seed banks of all plots contained high densities of cheatgrass seeds. The experimental design was a 2-x-2 factorial design, with two levels of inoculum and two levels of herbicide, in a randomized complete block. Two 8-by-10-m blocks were divided into four rows, and the inoculation treatment was randomly assigned to two rows in each block. Approximately half of each row had been sprayed with Roundup® the previous spring (isopropylamine salt of glyphosate). Within each row, two 1-by-1-m plots were established, one in the area that had received *Roundup*® and one in the unsprayed area. The inoculum was applied on October 30 and November 24, 1999. Substantial amounts of cheatgrass had emerged prior to October 30; these were removed from the plots prior to the first inoculation.

The number of cheatgrass seedlings in a 10-cm-diameter circular sample from within each 1-m^2 plot was counted on February 15 or February 20, 2000 and used to calculate the density of cheatgrass seedlings per m^2. The location of the sample within each plot was randomly selected using a 10-by-10 grid. The density of seedlings in each plot in February was also ranked as high or low to evaluate whether seedling densities in the circular samples reflected densities throughout the plots.

The coverage of perennial caespitose grasses in the plots was recorded on October 30, 1999 and again on February 23, 2000 to determine whether the inoculum negatively affected established bunchgrasses. The diameter of each bunchgrass clump was assigned to one of the following classes: 0-1 cm, 1-5 cm, 5-10 cm, and 10-15 cm. The canopy coverage of each bunch was estimated as the area of a circle with a diameter equal to the midpoint of the appropriate coverage class. Mean values for perennial grass cover in October and February were compared using a 2-tailed paired *t* test.

2.2.3. Results

The number of seedlings per sample in plots that were ranked as low in density ranged from 9 to 25 (mean 16.6 ± 5.4), whereas the number of seedlings per sample in the high density plots ranged from 44 to 118 (73.4 ± 28.5). This correspondence between seedling density in the 1-by-1-m plots and in the 10-cm-diameter samples suggests that a single sample per plot was representative of seedling density throughout the plot.

The densities of cheatgrass seedlings per square meter ranged from 1,146 to 15,021. Seedling densities were not lower in plots treated with *Pseudomonas* than in untreated plots, but they were significantly lower ($P = 0.001$) in plots that had been sprayed with Roundup® the previous spring (Table 1). The interaction between the *Pseudomonas* treatment and application of Roundup® was not significant ($P = 0.172$).

The coverage of perennial grasses February did not differ significantly from the coverage of perennial grasses in October for either the control plots ($P = 0.405$) or the inoculated plots ($P = 0.351$).

Table 1. Effects of herbicide and fall application of *Pseudomonas* on density of cheatgrass seedlings. Seedling densities per square meter were calculated on the basis of a 10-cm-diameter circular sample.

Mean number of seedlings/m^2 ± SD (range)		P level
Pseudomonas 6,588 ± 5,736 (1,146-15,021)	No *Pseudomonas* 6,031 ± 3,526 (1,655-11,457)	0.712
Roundup® 2,800 ± 2,057 (1,146-7,638)	No Roundup® 9,818 ± 3,624 (5,601-15,021)	0.001
Block A 6,954 ± 4,658 (1,655-15,021)	Block B 5,665 ± 4,781 (1,146-14,894)	0.400

2.2.4. Discussion

Inoculation with *Pseudomonas* did not adversely affect the growth of the perennial grasses in this experiment. Thus, it appears that biocontrol with *Pseudomonas* is appropriate for restoration of sites where the maintenance of native perennial grasses is desired; however, the inocula were not effective in suppressing cheatgrass either. Although *Pseudomonas* inocula have decreased the population size and growth of cheatgrass in growth chamber and field trials (Kennedy et al. 1991), in this experiment we did not find that the treatment had any effect on the density of cheatgrass seedlings, even in those plots that had been treated with *Roundup*® the previous spring. The densities of cheatgrass in the test plots were still fairly high (greater than 1,000 per m^2 in all cases), even after the first flush of seedlings, which had germinated prior to October 30, had been removed. Kennedy et al. 1991 tested moderate weed infestations. Since this study tested a heavy infestation, it might take multiple years for any inhibitory effects to appear. The question of whether the *Pseudomonas* inocula would be effective at sites with smaller seed banks is worth investigating.

We did not determine whether the test inoculum reduced the vigor of cheatgrass seedlings. It is possible that even if the size of the cheatgrass population did not decline, a reduction in cheatgrass vigor might give native perennial grasses a competitive advantage and reduce the impact of cheatgrass infestations at sites with native perennial grasses.

Kennedy et al. (1991) found that the negative effects of *Pseudomonas* inocula were more pronounced at Washtucna, WA, which receives less precipitation than Pullman and which received below-normal precipitation in the year of the test (annual precipitation in 1987 was 250 mm at Washtucna and 400 mm at Pullman). This suggests that water stress might enhance the susceptibility of cheatgrass to deleterious rhizobacteria and that biocontrol with *Pseudomonas* might be more effective in drier zones.

2.2.5. Literature cited

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